ACHIEVING ADVANCED MATURATION AND SPAWNING IN YELLOWTAIL *SERIOLA QUINQUERADIATA* BY THE MANIPULATION OF PHOTOPERIOD AND WATER TEMPERATURE

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ABSTRACT

Yellowtail *Seriola quinqueradiata* held in captivity begin maturing and spawning in late April to early May, while yellowtail in the wild spawn about 2 mo earlier. The 2 mo difference in spawning periods is significant, as the cultivated fry are smaller than those in the wild when they are released for stock enhancement. The purpose of this study was to develop techniques for obtaining eggs of yellowtail in captivity earlier than usual to coincide with the natural spawning season of yellowtail in the wild. The first experiments examined the effects of extended daylength (1800-2400; EDL) on ovarian maturation and the use of human chorionic gonadatropin (HCG) to induce final maturation and spawning. In 1991, 1992, and 1993, female broodstock were induced to mature more rapidly than those under natural photoperiod by extending daylength by 6 h for 28 ds (1991, 1992) or 20 ds (1993). Each year, the mean number of eggs from fish under EDL was higher than that of the control group. The results demonstrate that manipulation of daylength is an effective method for accelerating maturation in female yellowtail broodstock.

Further experiments in 1994-1995 and 1995-1996 examined the combined effects of photoperiod control on the maturation of yellowtail, namely, a short-day (SD) treatment of 1 mo followed by a long-day treatment and water temperature control (≥19 C) for one mo. In both years, the daylength was set to 8 h (8 L: 16 D) for 1 mo followed by a 10 h extension (18 L: 6 D) for the next mo under controlled water temperature. Female broodstock kept under controlled photoperiod and water temperature were induced to mature more rapidly than those maintained under natural conditions. However, neither controlled photoperiod nor water temperature alone was sufficient to induce maturation in yellowtail. After HCG injection, during both years, fish kept under EDL or warm temperature spawned earlier than usual in captivity. Consequently, photoperiod and water temperature manipulations are effective in accelerating maturation of female yellowtail broodstock to the point where fertilized egg production can be achieved by the induction of spawning using HCG.

INTRODUCTION

The wild population of yellowtail *Seriola qiunqueradiata* is one of the most valued fishery resources in Japan. However, the natural stock has steadily declined over the years. In 1978, the first program for broodstock management and production of the juveniles of yellowtail for stock enhancement was initiated by the Japan Sea-Farming Association (JASFA) to offset the decline. Due to the advancements in techniques for the induced spawning of broodstock and the rearing of larvae and juveniles, as many as 1 million juveniles/yr have been produced.

The spawning of wild yellowtail in the waters around Shikoku and Kyushu has been observed from late February to April (Umeda 1991). However, the spawning season of yellowtail reared in net cages under natural conditions at the JASFA Komame Station in Kochi Prefecture occurs about 2 mo later. Due to this delay in spawning in captivity, artificially-produced juveniles are much smaller than wild juveniles at the time of release, resulting in poorer survival. The project initiated by JASFA was designed to obtain eggs at an earlier period in order to release tagged juveniles close to the same size and same age as juveniles found in the wild.

There have been many investigations of controlling the natural spawning season in fish by manipulating environmental factors such as photoperiod and water temperature (Breton and Billard 1977; MacQuarrie et al. 1978; Whitehead et al. 1978; Beacham and Murray 1993). The present study focused on determining appropriate photoperiod and temperature regimens to result

in the production of spawned eggs at an earlier time than usual from captive yellowtail broodstock.

MATERIALS AND METHODS

Yellowtail Broodstock

Experiment 1 was conducted during 1991 and 1993. Yellowtail used as broodstock in 1991 (Table 1) were captured by set-nets in Komame inlet (Kochi Pref.) and reared on moist pellets (MP) (Mushiake et al. 1993) for about 2 yr in a floating rectangular net cage (10 x 5 x 6 m) at the Komame Station of JASFA. All females used for the experiments were marked individually by a personal identification tag (PIT) (Identification Devices Inc., USA), implanted into the dorsal muscle when they were transferred from the net cage into indoor spawning tanks (110 m^3). Fish used in the 1992 and 1993 experiments were transferred from a private farm in Ehime Prefecture to the Komame Station and fed on moist pellets for 1.5 yr as captive broodstock.

In experiment 2 , during 1994-1995 and 1995-1996, the adult yellowtail were captured by set-nets in the Komame inlet and fed moist pellets (MP) or commercial soft-dry pellets (SDP: Sakamoto Fish Feed Co. Ltd., Chiba, Japan) (Mushiake et al. 1995) during 1994 and 1995, respectively, at Komame Station (Table 1). The fish were reared in net cages (10 x 10 x 6 m) for a period of 8 mo and transferred to indoor spawning tanks (65 m^3) for spawning on 14 November in 1994 and 1995. All females were marked individually by PIT.

Experimental Rearing Conditions

Table 1 shows a summary of the two different groups in experiment 1 and the four groups in experiment 2. Each group in experiment 1, either the EDL-treated or control, consisted of 20 (1991) or about 10 (1992, 1993) individuals. The experiments were begun by placing the fish in the spawning tanks (110 m^3). All fish were kept under natural lighting conditions from sunrise to 1800. The group exposed to EDL received

Table 1. Details of the yellowtail broodsotock used for the experiments

Year	Group No.	Origin	No. of fish (M:F)[*2]	Diet[*3]	Fork length ±SD (cm)	Body weight ±SD (kg)	Condition factor ±SD	Rearing condition Photoperiod[*4]	Water temperature[*5]
Experiment 1									
1991		Wild-1	20 (12:8)	MP	82.5±3.3	11.38±1.53	20.22±1.21	controlled-1	natural
		Wild-1	20 (12:8)	MP	82.4±2.9	11.42±1.60	20.24±1.34	natural	natural
1992		Wild-2	10 (6:4)	MP	77.8±2.1	9.31±0.83	19.79±1.36	controlled-1	natural
		Wild-2	9 (6:3)	MP	76.6±1.3	8.70±0.54	19.38±1.46	natural	natural
1993		Wild-2	12 (6:6)	MP	76.3±1.9	8.51±0.67	19.17±0.70	controlled-1	natural
		Wild-2	12 (6:6)	MP	73.8±1.9	7.78±0.66	19.36±1.05	natural	natural
Experiment 2									
1995	1	Wild-1	6 (3:3)	MP	81.9±1.7	10.36±1.24	18.65±1.06	controlled-2	controlled
	2	Wild-1	6 (3:3)	MP	81.6±1.4	10.14±1.86	18.67±1.15	controlled-2	natural
	3	Wild-1	6 (3:3)	MP	81.9±1.6	10.28±1.26	18.71±1.04	natural	controlled
	4	Wild-1	6 (3:3)	MP	81.8±1.8	10.31±1.45	18.83±1.11	natural	natural
1996	1	Wild-1	12 (6:6)	SDP	80.7±2.1	9.86±1.22	18.79±1.27	controlled-2	controlled
	2	Wild-1	12 (6:6)	SDP	81.1±1.9	9.95±1.71	18.66±1.34	controlled-2	natural
	3	Wild-1	12 (6:6)	SDP	81.0±1.9	9.77±1.68	18.39±1.08	natural	controlled
	4	Wild-1	12 (6:6)	SDP	80.9±1.7	9.91±1.49	18.71±1.42	natural	natural

[*1] Wild-1: Captured by set-net fishery and reared for 2 years, Wild-2: Captured at juvenile stage and reared for 4 years in all.

[*2] M: male, F: female.

[*3] MP: Moist pellets (formula feed prescribed by National Research Institute of Aquaculture, Japan + raw fish (1:1)), SDP: Commercial soft dry pellets (Sakamoto Fish Feed Co. Ltd., Chiba, Japan).

[*4] Controlled-1: Extended daylength treatment (EDL) from 18:00 to 24:00, controlled-2: short-day treatment for one month followed by EDL treatment for the next one month.

[*5] Controlled: Water temperature was kept at a minimum of 19 C.

additional lighting provided by two tungsten flood lights (200 W/l) supported above each tank from1800 to 2400. The EDL treatment was initiated on the day the fish were stocked into each experimental aquarium, and continued for 28 (1991, 1992) or 20 (1993) days at which time they were injected with human chorionic gonadotropin (HCG) to induce final maturation and spawning. The water temperature in experiment 1 was maintained around 19 C for the duration of the experiment.

In experiment 2, the broodstock in group 1 were maintained under both controlled photoperiod and water temperature. In group 2 the photoperiod and in group 3 the water temperature was controlled, respectively. No environmental manipulation was provided for fish in group 4. Photoperiod manipulation consisted of a short-day (SD) treatment followed by EDL treatments. The SD treatment was performed by spreading a matted black sheet (light transmittency 0%) over the surface of each tank from 1700 in the evening until 0900 the next morning (8 L-16 D). The SD treatment was initiated on d 3 (17 November: d 0 in Fig. 2) after transferring the fish into the indoor spawning tank, and continued for either 31 d (until 18 December 1994) or 32 d (until 19 December 1995). The EDL treatment commenced on the day following the termination of the SD treatment and continued for either 32 d (until 19 January 1995) or 34 d (until 22 January 1996). The EDL treatment was attained by extending the daylength 1 h every 3 or 4 d until daylength reached 18 h. The long photoperiod (18 L) was maintained from 19 January 1995 and 22 January 1996, throughout the spawning period.

The water temperature of the temperature-controlled tanks (1 and 3) in 1994-1995 and also 1995-1996 was kept at a minimum of 19 C by inflow of sea water heated with thermostatic devices. In groups 2 and 4, the water temperature was allowed to fluctuate naturally.

Examination of Ovarian Maturation

Ovarian tissue was sampled by inserting a cannula into the genital pore of fish which had been individually identified by their PIT tag. For each fish, the diameters of 100 sampled oocytes were examined under a stereoscopic microscope (Nikon) and the mean oocyte diameter was calculated. Statistical analysis comparing the mean oocyte diameter between experimental groups was performed by *t*-test.

In experiment 1, the state of maturation in the females was assessed by cannulation three times in 1991 and 1992, just prior to distributing the fish into the spawning tanks (d 0), and on d 14 and d 28 after the start of experiments in both the EDL and control groups. In 1993, maturation examinations were carried out on d 0, d 10 and d 20.

In experiment 2, the stage of ovarian maturation was examined four times: just prior to distributing the fish into the spawning tanks on d 31 and d 32 (when the SD treatment was completed), on d 63 and d 66 (when the EDL treatment was completed), and on d 76 and d 97 (when fish were injected with HCG) in 1995 and 1996.

Induced Spawning by Hormone Injection and Evaluation of Egg Quality

In experiment 1, in order to induce spontaneous spawning in the indoor spawning tanks, HCG was injected at a dosage of 600 IU/kg BW into the dorsal muscle of both sexes, on d 28 (31 March 1991; 4 April 1992), or d 20 (25 March 1993) of EDL treatment.

In experiment 2, HCG was injected on d 76 in 1995 (1 February) and d 97 in 1996 (19 February). About 2 d after the administration of HCG in both experiments, fish began to spawn. Eggs were collected each day from 1700 to 0900 for as long as the fish continued to spawn. The number of eggs produced per fish/d was estimated by counting the number of eggs in a volume of 1 ml.

The diameters of 50 buoyant eggs, percent fertilization and the number of eggs having more than one oil droplet (abnormal eggs) were examined using a profile projector (Nikon). Percent hatching was also determined by estimating the numbers of larvae in each net. The percentage of normal larvae was estimated by counting the deformed and abnormal larvae (larvae having more than one oil droplet or having unusual oil deposition).

RESULTS

Ovarian Maturation by the EDL Treatment

The changes in oocyte diameters with time in all test groups in experiment 1 (1991-1993) and 2 (1994-1995, 1995-1996), are summarized in Fig. 1 and 2, respectively. Mean oocyte diameters at the start of experiment 1 ranged between 562 and 584 μm as shown in Fig. 1. In 1991 and 1992, mean oocyte diameters in the EDL group on d 28 were significantly (P<0.01) larger than those in the control groups. In 1993, the oocyte diameters in the EDL group on d 20 were also significantly (P<0.01) larger than those obtained from the control group.

In experiment 2, conducted in 1994-1995 and 1995-1996 (Fig. 2) the mean oocyte diameters at the beginning of experiments ranged from 309 to 314 μm and 339 to 347 μm, respectively. In 1994-1995, the mean oocyte diameters in the group 4 (that did not receive any environmental control) were 343 μm on d 31, 438 μm on d 63, and 504 μm on d 76 of the experiment. Mean

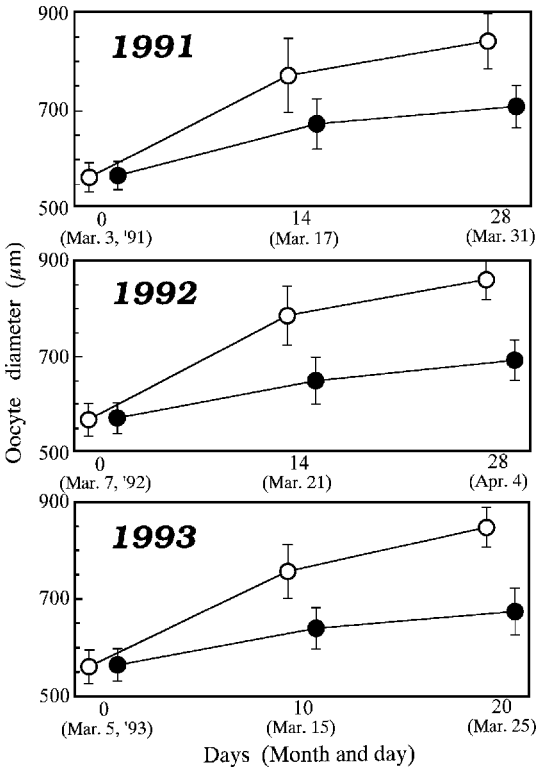

Figure 1. Changes in mean oocyte diameter of yellowtail in experiment 1. Vertical lines represent the standard error. ❍, EDL treatment; ●, control.

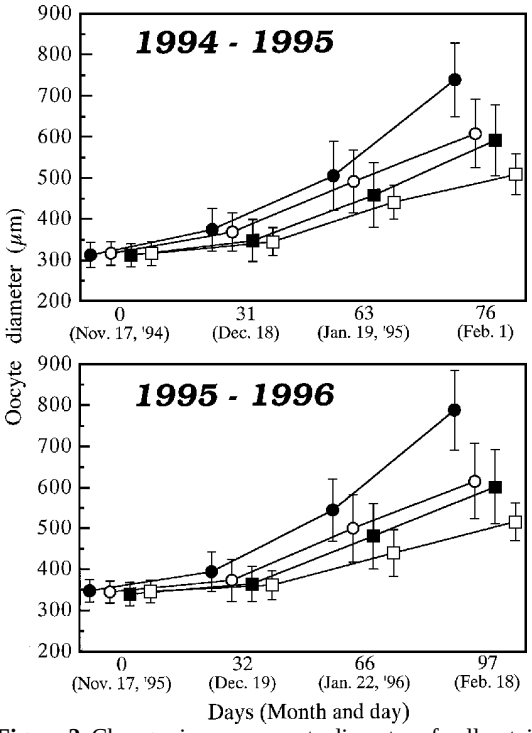

Figure 2. Changes in mean oocyte diameter of yellowtail in experiment 2. Vertical lines represent the standard error. ●, group No. 1; ❍, No. 2; ■, No. 3; and ❑, No. 4.

oocyte diameters in fish from the photoperiod and water temperature-controlled group (1) were 373, 503, and 735 μm on d 31, d 63, and d 76, respectively. The mean oocyte diameters of fish from group 1 on d 76 was significantly larger (P<0.01) than those obtained from group 4. The mean oocyte diameters of females from either the photoperiod (2) or water temperature (3) controlled groups were intermediate to those of groups 1 and 4. In 1995-1996, the mean oocyte diameters were 359, 436, and 511 μm in group 4 and 393, 542, and 784 μm in group 1 on d 32, d 66, and d 97, respectively, indicating a significant difference (P<0.01) in the state of maturation between the two groups. The trend of increasing mean oocyte diameters in the other two groups (2 and 3) was similar in both years.

Induced Spawning

The results of induced spawning trials of yellowtail broodstock injected with HCG in experiment 1 and 2, are summarized in Tables 2 and 3, respectively. In experiment 1, the numbers of eggs produced in the EDL treatment during 1991 and 1993 were significantly (P<0.01) higher

Table 2. Induced spawning results of yellowtail injected with HCG in experiment 1

Year		1991		1992		1993	
Test group		EDL[*1]	Control	EDL	Control	EDL	Control
Spawning period		Apr.3-Apr.15	Apr.3-Apr.17	Apr.7-Apr.16	Apr.7-Apr.16	Mar.28-Apr.9	Mar.28-Apr.9
Spawning days		13	15	10	9	13	13
Eggs							
Eggs produced/fish	($X10^3$)	2029.8[*2]	958.2	2139.1[*2]	1058.9	2246.3[*2]	987.1
Buoyant eggs/fish	($X10^3$)	1657.1[*2]	607.6	1418.7[*2]	628.1	1680.1[*2]	605.8
Rate of buoyant eggs	(%)	81.6[*3]	63.4	66.3	59.3	74.8	61.4
Rate of fertilized eggs	(%)	77.0[*3]	54.9	57.1	49.3	58.4	49.1
Hatched larvae							
Total larvae obtained from total eggs	(%)	42.3[*2]	18.2	42.2[*2]	21.3	43.6[*2]	25.6
Normal larvae obtained from total eggs	(%)	28.4[*2]	10.7	31.5[*2]	11.7	32.4[*2]	15.2

[*1] EDL: extended daylength treatment.
[*2] Significantly different ($p<0.01$) as compared with the result of the control in the same year (t-test).
[*3] Significantly different ($p<0.05$) as compared with the result of the control in the same year (t-test).

Table 3. Induced spawning results of yellowtail injected with HCG in experiment 2

Year		1994 - 1995				1995 - 1996			
Test group		No. 1	No. 2	No. 3	No. 4	No. 1	No. 2	No. 3	No. 4
Spawning period		Feb.3-Feb.9	-	-	-	Feb.21-Mar.3	-	-	-
Spawning days		6	0	0	0	9	0	0	0
Eggs									
Eggs produced/fish	($X10^3$)	418.0	0	0	0	1541.7	0	0	0
Buoyant eggs/fish	($X10^3$)	155.7	-	-	-	759.2	-	-	-
Rate of buoyant eggs	(%)	37.2	-	-	-	49.2	-	-	-
Rate of fertilized eggs	(%)	14.8	-	-	-	66.6	-	-	-
Hatched larvae									
Total larvae obtained from total eggs	(%)	4.2	-	-	-	24.7	-	-	-
Normal larvae obtained from total eggs	(%)	1.8	-	-	-	23.6	-	-	-

than those from the control groups. There was also a significant difference ($P<0.01$) in the number of normal larvae obtained between the two groups.

In experiment 2, only the fish in test group 1 spawned on d 2 after administration of HCG in 1994-1995 and in 1995-1996. In 1994-1995, after the injection of HCG, the broodstock began to spawn on 3 February and spawned daily until 9 February. They produced 418.0 x 10^3 eggs per fish, 37.2% of which were buoyant. In 1995-1996 the fish injected with HCG began to spawn on 21 February, and spawned daily from 24 February to 3 March. They produced 1541.7 x 10^3 eggs/ fish, 49.2% of which were buoyant.

DISCUSSION

Changes in the mean oocyte diameter with time in experiment 1 (Fig.1) clearly indicate that ovarian maturation was accelerated by the EDL treatment. All data concerning egg quality indicated that the broodstock exposed to EDL treatment produced eggs that were superior to those in the control groups. This result demonstrates that the maturity of yellowtail broodstock can be manipulated by EDL to result in large numbers of eggs of suitable quality at a time that should result in the production of seedlings of appropriate size for use in stock enhancement activities.

As shown in Fig. 2, changes in mean oocyte diameters of broodstock in experiment 2 indicate that ovarian maturation in yellowtail was accelerated by manipulating both photoperiod and water temperature. The broodstock of all groups were injected with HCG on 1 February (on d 76) and 19 February (on d 97) in 1994-1995 and 1995-1996, respectively. The fish having mean oocyte diameters around 700 μm could be spawned by HCG injection, although the quantity and quality (percent buoyancy, fertilization, and hatching) were low. HCG-treated fish that possess mean oocyte diameters around 800 μm (1995-1996) responded by spawning a larger number of eggs that were decidedly of better quality. Therefore, it was concluded that the success of accelerated egg production from yellowtail broodstock would also depend on administering HCG at the appropriate state (mean oocyte diameter of 800 μm) of maturity.

Spawning results (number of eggs and egg quality) of group 1 in 1995-1996 were superior to those in 1994-1995, but not as good as those reported previously from other culture activities (Mushiake et al. 1995). However, for the stock enhancement of yellowtail, in order to release juveniles of similar size to those in the wild, egg production earlier than the normal captive spawning period is required. Further research is necessary to improve the egg quality of from the advanced spawning yellowtail when maintained under controlled photoperiod and water temperature conditions.

For pink salmon *Oncorhynchus gorbuscha* (Beacham and Murray 1988, 1990), it has been suggested that acceleration of maturation is more likely to be achieved through manipulation of photoperiod rather than water temperature. The distinction between which environmental parameter is more important could not be discerned for the yellowtail broodstock, as the mean oocyte diameters from (photoperiod-controlled) and (water temperature-controlled) did not differ significantly from each other.

Although the mechanism by which ovarian maturation is accelerated remains to be clarified, it was found that yellowtail kept under controlled photoperiod and water temperature were able to spawn in February, 2 mo earlier than those held under ambient conditions. These results indicate that yellowtail juveniles can be produced earlier than usual at the JASFA Yashima Station, Kagawa Prefecture. In 1996, seed production was conducted and tagged juveniles were released into the sea 2 mo earlier than previously reported. The released fish, which were similar in size to their counterparts in the wild, showed a high percent recovery (12.9%) compared with the usual 0.2–3.1% of fish produced and released during the usual time near the eastern part of the Seto Inland Sea (unpublished data).

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